EXAMPLES

Example 1

Modification of the 3' ends of an RNA library.

On the day of use, a 200mM stock solution of sodium periodate (Sigma-Aldrich, St. Louis, MO) was prepared in nuclease-free water (Ambion, Austin, TX). To prepare a working solution, 10µl of 200mM sodium periodate was added to 990 µl of 10mM sodium acetate. 100 µg of total RNA from human liver (Stratagene, La Jolla, CA) in nuclease-free water was mixed with an equal volume of 2mM sodium periodate in 10mM sodium acetate, incubated for 60' on ice and protected from light. The RNA was purified using an RNEASY® column (Qiagen,Valencia, CA) according to the manufacturer's protocol. The integrity of the modified RNA was verified by agarose gel electrophoresis and staining with SYBR® Gold (Molecular Probes, Eugene, OR). First strand cDNA was synthesized from lOpg of modified and un-modified human liver RNA templates using SUPERSCRIPT® II RNaseH reverse transcriptase (Invitrogen, Carlsbad, CA) according to the manufacturer's specifications, in the absence or presence of dT_{24} primer. RNA templates were removed by alkaline hydrolysis in 0.6M NaOH, 4mM EDTA (2x) at 37°C for 15' followed by neutralization with 6M acetic acid (20x). cDNA was then purified on MINELUTE® columns (Qiagen,Valencia, CA) and eluted in lOpl buffer ES following the manufacturer's directions. DNA electrophoresis was carried out in a 1 % agarose 0.5 xTBE gel, stained with SYBR® Gold and viewed with a Digital Image Station (Kodak, Rochester, NY).

Results

The results of this experiment are shown in Figure 3. Lanes 2 and 4 demonstrate the ability of both treated (lane2) and untreated (lane 4) RNA to act as templates for cDNA synthesis with oligo T primers. On the other hand, although there is extensive cDNA synthesis in the absence of exogenous primers

APPENDIX C